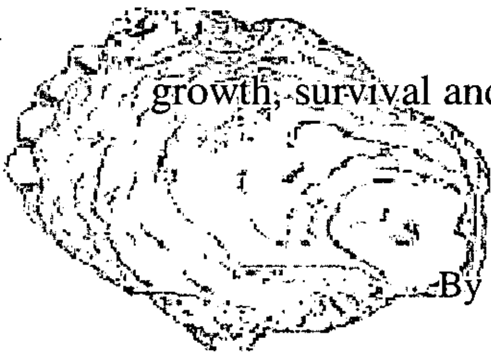
COMMERCIALISATION OF TRIPLOID SYDNEY ROCK OYSTERS, *SACCOSTREA GLOMERATA*, IN NEW SOUTH WALES

growth, survival and meat condition



By

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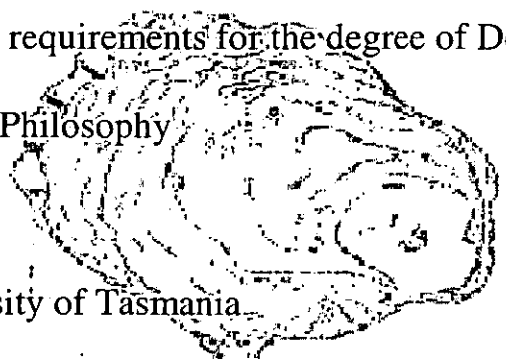
Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy



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CERTIFICATE

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ABSTRACT

To aid the commercialisation of triploid technology for Sydney rock oysters (*Saccostrea glomerata*) in NSW, triploids and diploids were evaluated for growth, mortality and meat condition on commercial oyster farms throughout the state. After 2½ years, triploids were on average 30.7% heavier and 8.6% larger in shell height than same parent diploids. Mortality of triploids was significantly lower ($p < 0.01$) or not significantly different ($p > 0.05$) from that of diploids at 12 of the 13 sites. Average cumulative mortality after 2½ years across the 13 sites was 28% for diploids compared to only 16% for triploids. Performance of triploids over diploids varied considerably between sites. Wild-caught diploids had lower growth rates and higher mortality than both diploid and triploid hatchery stock.

At seven sites where oysters were exposed to the parasite *Mikrocytos roughleyi* (cause of "winter mortality"), triploid Sydney rock oysters survived the disease better than diploids. Cumulative mortality of diploids during the second winter/spring at these sites was 35% compared to only 12.2% for triploids.

Meat condition of diploids and triploids varied between the five sites throughout NSW. Over the final year on leases, ploidy, month and the ploidy*month interaction had a significant effect on meat condition at all sites except for ploidy at the southern, Lake Pambula site. From March to December (autumn to the first month of summer) condition indices of triploids were greater, or not significantly different from those of diploids at all sites. Triploid Sydney rock oysters were susceptible to brown discolouration of the gonad surface. Discolouration occurred in localised areas of the gonad and was not correlated to

condition index except for triploids at Lake Pambula. As discolouration was less noticeable during cooler months of the year, it coincided with the generally superior condition of triploids relative to diploids during winter and spring, so that triploids remain a viable winter crop for farmers throughout NSW.

After two years, an experiment in Port Stephens showed triploid oysters from two initial size grades were heavier and larger than equivalent size grades of same parent diploids ($p < 0.05$). Initial size grade had a significant effect on final mean whole weight and shell height for both ploidy types ($p < 0.05$). There was no significant difference in the final percentage triploidy between small and large grade triploids. A large proportion of diploid/triploid mosaicism was detected in adult triploid oysters.

To determine if improvements in growth of a selected oyster line (L2) were additive to the faster growth of triploids, the performance of diploid and triploid selected and control oysters (four oyster lines) was compared. After a grow-out period of 21 months both mean whole weights and shell heights were in the order: L2 triploids > control triploids > L2 diploids > control diploids. A significant ($p < 0.05$) site*line interaction effect on whole weights and shell heights was detected. Growth improvements from selective breeding and triploidy were found to be additive with L2 triploids being 63% heavier than control diploids after 21 months grow-out. In this experiment, the type of oyster had no effect on final condition index, percent cavity volume, percent shell weight or cumulative mortality. Both diploid and triploid selected oysters had significantly ($p < 0.05$) higher whole weight to shell height ratios than diploid and triploid control oysters.

Triploid Sydney rock oysters were shown to outperform diploids throughout NSW in terms of growth, survival and meat condition. The demand for both diploid and triploid Sydney rock oyster spat is now increasing and the demonstrated ability to combine the growth advantages of triploidy with selective breeding will no doubt increase the demand for hatchery spat further. However, commercial uptake of triploid technology will rely on overcoming the problems of early larval and spat mortality of hatchery reared Sydney rock oysters to ensure continuity of supply to farmers.

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THE NEW SOUTH WALES' OYSTER INDUSTRY AND THE POTENTIAL OF TRIPLOIDS

1.1 OVERVIEW OF OYSTER AQUACULTURE IN AUSTRALIA

The Australian aquaculture industry is a rapidly developing industry valued at around $680 million annually (ABARE 2001). With the combination of developing culture technologies and declining natural fisheries stocks, the value of annual national aquaculture production increased by 73% in the five years between 1994-95 and 1999-00 (ABARE 1997; ABARE 2000).

Production figures for edible oyster aquaculture and species farmed in each state are shown in Table 1.1. New South Wales (NSW) aquaculture is dominated by the long-established Sydney rock oyster, *Saccostrea glomerata* (Gould 1850) (formerly *S. commercialis*; Buroker et al. 1979; Anderson and Adlard 1994) industry with annual production of 5 024 t valued at $26 million in 1998-99 (NSW Fisheries 1999). The other edible oyster species farmed in NSW since its production was legalised in 1990 is the introduced Pacific oyster, *Crassostrea gigas,* Thunberg with production of 290 t valued at $1.5 million in 1998-99 (NSW Fisheries 1999). The contribution of oysters to the NSW economy is however, declining with a drop from 86% of the value of total aquaculture production in 1990-91 to 69% in 1999-2000 (Brown et al. 1997; ABARE 2001).

The Pacific oyster is the major oyster species farmed in Tasmania (4748 t in 1999-2000) and South Australia (2494 t in 1999-2000) (ABARE 2001) having been introduced to both states to establish an oyster industry (Ayres 1991). Flat oysters, *Ostrea angasi*, are cultured on a small scale in Tasmania. With a total edible oyster production of 143 t in 1999-2000 (ABARE 2001), the tropical Queensland waters are host to several oyster species; the Sydney rock oyster is the major species farmed with smaller numbers of milky oysters, *Saccostrea cucullata*, and black lip oysters, *Striostrea mytiloides*, produced. In Western Australia (WA), there is small-scale commercial production of Sydney rock oysters and flat oysters whilst in Victoria limited numbers of Pacific oysters are cultured in salt ponds (Brown et al. 1997). Figures for edible oyster production in WA, Victoria and the Northern Territory are not available (ABARE 2001). There is also a substantial pearl oyster industry in WA valued at $190 million in 1999-2000 (ABARE 2001).

International trade in oysters has grown over the last decade with imports world-wide of 26 900 t ($US108 million) in 1998 compared to 18 300 t in 1988 (FAO 2000). With large quantities remaining unrecorded in Asia, world production of oysters in 1998 was at least 3.5 million t (FAO 2000) while aquaculture production of edible oysters in Australia between 1999-2000 was only 12 969 t (Table 1.1). This expanding international market represents an unfilled niche for producers of the Sydney rock oyster in NSW. The Sydney rock oyster is considered to be a gourmet oyster but is presently not exported in large quantities; only 1 t of oysters was exported from NSW in 1998-99 compared to 49 t from Tasmania (ABARE 2000).

Table 1.1

Edible oyster production in Australia by state, 1999 - 2000[1].

State or Territory	**Production**	**Value**
species farmed	**(t)**	**(AUD $ million)**
New South Wales	5 584	28.8
Sydney rock oyster, *Saccostrea glomerata*		
Pacific oyster, *Crassostrea gigas*		
Flat oyster, *Ostrea angasi*		
Northern Territory	not available	not available
Queensland	143	0.65
Sydney rock oyster, *Saccostrea glomerata*		
Milky oyster, *Saccostrea cucullata*		
Black lip oyster, *Striostrea mytiloides*		
South Australia	2 494	9.3
Pacific oyster, *Crassostrea gigas*		
Tasmania	4 748	13.2
Pacific oyster, *Crassostrea gigas*		
Victoria	not available	not available
Western Australia	not available	not available
Total	12 969	51.9

[1]ABARE 2001

1.2 THE SYDNEY ROCK OYSTER INDUSTRY IN NSW

The Sydney rock oyster industry is amongst the oldest in Australia with the first oysters farmed around the 1870s in NSW and Queensland (Roughley 1922; Smith 1981/82). The age of the industry in NSW may account for its large size (around 43% of Australian edible oyster production in 1999-2000 (Table 1.1, ABARE 2001)) as well as for the persistence of early culture techniques such as stick culture. The industry is largely based on traditional wild-caught oyster seed rather than hatchery produced seed; i.e. juvenile oysters are caught on tarred hardwood sticks and either on-grown or transferred to mesh trays until a marketable size. More recently, some NSW farmers have begun to specialise in production and sale of wild-caught single seed; young oysters settled on plastic collectors or sticks are removed for ongrowing in mesh cylinders, trays or baskets. Single seed culture, although requiring a greater initial investment and labour cost, produces more marketable cup-shaped oysters (Nell 1993). Wild-caught single seed or "spat" are cheaper to produce than hatchery produced spat; however, initial mortality rates at grow-out are often higher in wild-caught spat due to shell damage during removal from collectors. Hatchery production of spat enables control over parent stock and larval production for breeding programs as well as a year-round supply of spat. At the commencement of this study, there were no commercial bivalve hatcheries operating in NSW. However, during the course of this research a commercial hatchery was established at Brooms Head in Northern NSW to produce both finfish and oysters. Since the establishment of the NSW Fisheries hatchery at Port Stephens, production of Sydney rock oysters has been subject to periodic outbreaks of "mass mortality" of spat (< 2.0 mm) with up to 95% of spat lost to the disease (Heasman et al. 2000). Disease problems during the early to mid larval phase (2 – 8 day old larvae) have also been a problem (Heasman et al. 2000) and have recently become a major limiting factor in reliable production of Sydney rock oysters at both the NSW Fisheries hatchery and the commercial hatchery at Brooms Head. Production of oysters at the Brooms Head hatchery was discontinued in 2000. Research into the cause of these problems is ongoing with trials using 'probiotics' (i.e. beneficial bacteria) producing promising results with early larvae (J. A. Nell pers. comm. 2000). Hatchery operators are also looking at ways to minimise the likelihood of outbreaks of the diseases occurring in both larvae and spat. Improvements in hatchery hygiene, alternate settlement methods and breeding early in the season are areas under investigation.

1.3 COMPETITION FROM THE PACIFIC OYSTER INDUSTRY

Sydney rock oysters generally take from 3 to 4 years to reach marketable size (Nell 1993) compared to 1½-3 years for Pacific oysters in Australia (Graham 1991). Besides being faster growing, Pacific oysters cultured in Tasmania are marketable for a longer period than the Sydney rock oyster due to cooler temperatures limiting spawning activity. Oysters in Australia are most marketable prior to spawning or when they have a high meat "condition index". The Sydney rock oyster generally spawns from summer to autumn, so is frequently unmarketable (or of poor meat condition) during the winter and spring months. In NSW, the Pacific oyster spawns earlier than the Sydney rock oyster and attains its best meat condition in late winter to spring (Nell and Mason 1991).

The mechanism by which Pacific oysters were introduced to NSW waters is poorly understood. Small numbers of Pacific oysters were found in Pambula River on the south coast of NSW in 1967 but were not reported again in NSW until 1973 when they were discovered in several central to south coast estuaries. It seems likely that the small numbers reported during the 1970's made their way into NSW estuaries as larvae via either ocean currents or vessels from established southern populations. However, the nature of the spread of Pacific oysters in Port Stephens, first recorded in 1985, has prompted speculation particularly from within the oyster industry that they were intentionally introduced to this estuary (Ayres 1991). The introduction of the Pacific oyster to NSW waters has had severe consequences for local farmers. Pacific oysters, which settle in large numbers in some estuaries, are classed as a noxious fish throughout NSW, with the exception of Port Stephens (Fisheries Management (General) Regulation 1995 (NSW) - section 229). As such it is illegal to possess Pacific oysters in NSW estuaries other than Port Stephens (Fisheries Management Act 1994 (NSW)- section 211). The increased handling time and costs involved in removing Pacific oysters combined with successful marketing strategies for single seed Pacific oysters produced in other states are steadily encroaching on traditional Sydney rock oyster markets. For example, in 1990-91 Pacific oyster production in Australia (by weight) was equivalent to 38% of Sydney rock oyster production (1663 and 4326 t respectively; Graham 1991). In 1997-98 Pacific oyster production had increased to 82% of that of Sydney rock oysters (4184 and 5089 t respectively (NSW Fisheries 1999; ABARE 2000)).

1.4 DISEASES OF THE SYDNEY ROCK OYSTER

The NSW oyster industry does not only have to contend with competition from the Pacific oyster industry, which is based on a faster growing oyster that maintains its meat condition for a greater proportion of the year. In recent years, farmed Sydney rock oysters have also been subject to a number of disease and health problems which have lowered the public image and therefore the market demand for Sydney rock oysters as well as affecting production levels (Nell 1999). Sydney rock oysters are susceptible to three major diseases: mudworm, *Polydora websteri* (Nell and Smith 1988), winter mortality caused by the protistan parasite, *Mikrocytos roughleyi* (Farley et al. 1988) and QX caused by the haplosporidian parasite, *Marteilia sydneyi* (Perkins and Wolf 1976). Recent evidence points to the possible misclassification of winter mortality in the genus *Mikrocytos*; Cochennec-Laureau et al. (2001) suggest that it may instead belong to the genus *Bonamia*. These diseases can result in heavy losses of Sydney rock oyster stock (up to 95%) and increased handling and labour costs. The latter two do not affect Pacific oysters although it is possible Pacific oysters may act as a carrier of either disease. Pacific oysters in the USA act as a carrier and reservoir host of another microcell disease related to winter mortality, *Mikrocytos mackini* (Bower et al. 1994). Pacific oysters have also been reported to have carried *Haplosporidium spp* parasites from Japan and Korea to the USA (Burreson et al. 2000). Recent ultrastructural analysis of *M. roughleyi* indicates that it may be a mis-classified haplosporidian (Cochennec-Laureau et al. 2001). While both winter mortality and QX do not affect consumers of shellfish, they do detract from the appearance (Fig. 1.1) and therefore the marketability of the oysters.

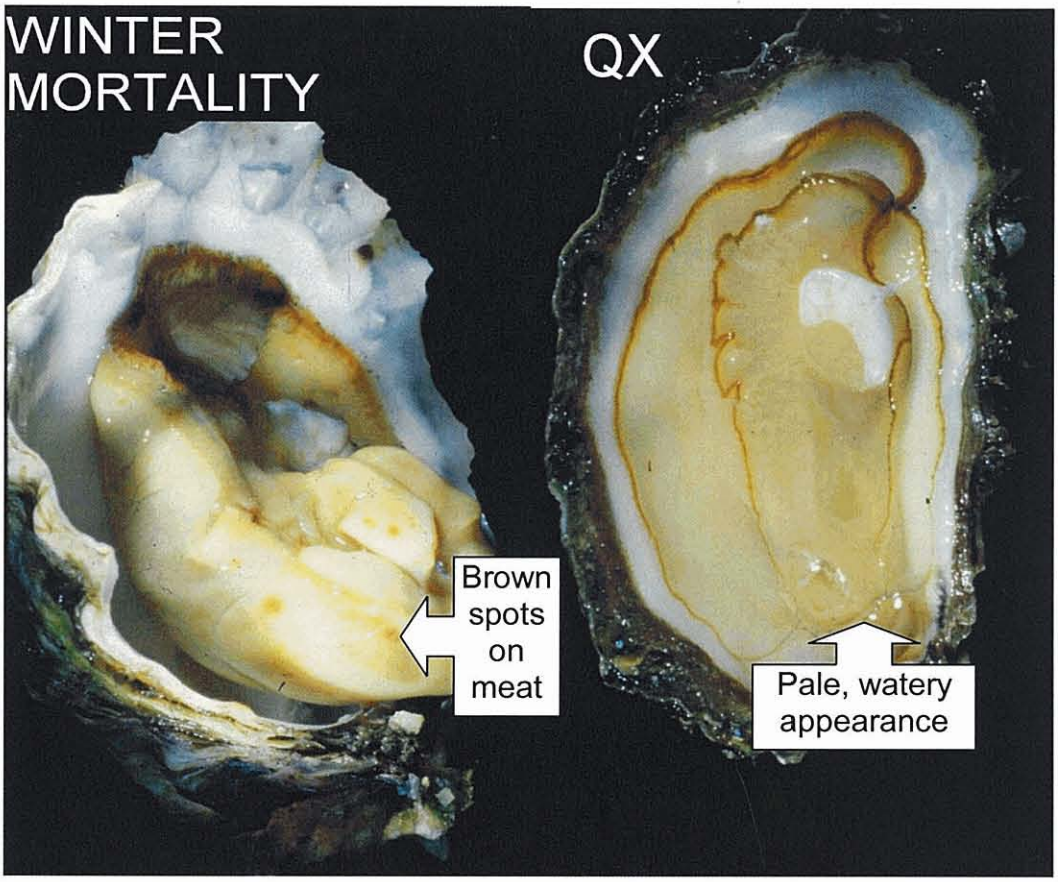

Figure 1.1. Symptoms of the diseases winter mortality and QX in Sydney rock oysters.

M. roughleyi infestation of oysters (winter mortality) is characterised by the appearance of yellow to brown lesions on the labial palps, mantle, gills, gonad and/or adductor muscle (Roughley 1926; Wolf 1967). Oysters eventually lose the ability to remain closed when exposed to air at low tide. *M. sydneyi* infestation (QX) attacks the digestive diverticulum tubules of the oyster (Perkins and Wolf 1976) affecting food absorption. This gives the oyster a pale watery appearance that is not well accepted by Australian consumers of oysters where a high meat condition index of oysters is preferred. In addition, infected stock is obviously unmarketable when a high proportion is moribund. The two diseases remained geographically separate up until 1994; winter mortality occurring in the cooler, mid to southern half of NSW and QX in the northern oyster farming estuaries (Fig. 1.2). In 1994 the range of QX extended south to Georges River in Sydney causing large losses of stock for oyster farmers in the region who also had to contend with winter mortality. Production continued to be severely affected in Georges River until the industry's total demise in 2001. It is possible that QX may spread to other estuaries previously thought to be out of the geographic "range" of the disease.

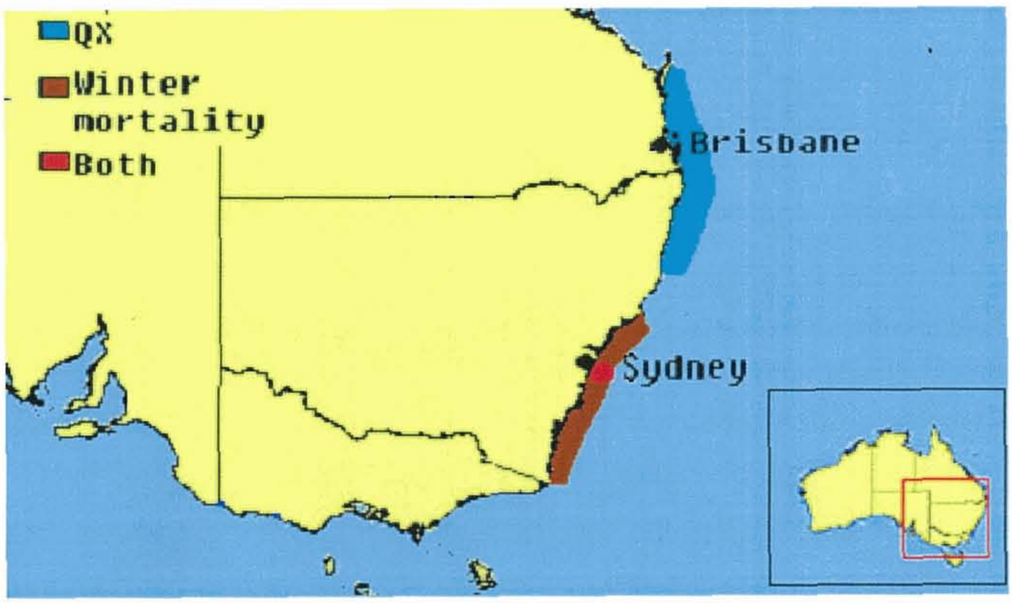

Figure 1.2 Current range of QX and winter mortality, two diseases that affect Sydney rock oysters

Mudworm (*P. websteri*) can result in heavy losses to the oyster farmer, both from sometimes high mortality rates of stock as well as through unmarketability of infested oysters. Oysters infested by mudworm deposit a mud blister of conchiolin to cover the wastes produced by the worm. The mud inside this blister eventually becomes black, anoxic and smells strongly of hydrogen sulphide. Infested oysters often have poor meat condition and may be more easily affected by other stresses. Tissue in contact with the mud blister eventually becomes yellow in colour (Nell and Smith 1988).

1.5 TRIPLOID OYSTERS

If the Sydney rock oyster industry is to remain competitive against the expanding Pacific oyster industries there is a need to develop new marketing strategies and production technologies. After two generations of selection in Georges River, a selective breeding program for Sydney rock oysters has been successful, achieving a reduction in mortality from 91% of controls to 56% of oysters bred for resistance to QX (Dr J. A. Nell, NSW Fisheries, unpublished data). Sydney rock oysters produced from a breeding program for faster growth in Port Stephens, NSW were 18% heavier than control oysters after two generations of selection (Nell et al. 2000). Although effective, selective breeding for faster growth of Sydney rock oysters is a slow process taking two years for each generation to reach a suitable breeding size. Another potential and more immediate means of dealing with slower growth as well as with seasonality of meat condition is the introduction of triploid oyster culture. Triploid oysters are oysters with three sets of chromosomes (two maternal and one paternal set or 3n=30 chromosomes in somatic cells) instead of the normal two sets in diploids (20 chromosomes). Induction of triploidy in molluscs results in functional sterility. Triploids rarely reach spawning condition and although triploid gametes can be fertilised, fecundity and survival of progeny are both extremely low. Factorial matings of triploid and diploid Pacific oysters (by artificially removing gametes) conducted by Guo and Allen (1994a) resulted in less than 0.05% survival of triploid cross offspring to 2 months post-fertilisation.

Fecundity of triploid female Pacific oysters in the same study was only 2% of that of diploids. Fecundity of male triploids may be even more compromised than female triploids (Allen 1994; Guo and Allen 1994a). Spermatogenesis is retarded beyond differentiation of spermatocytes (at prophase I) with development of few or no spermatids or spermatozoa (Allen and Downing 1990; Cox et al. 1996; Kiyomoto et al. 1996). In a number of species, the reduction in gametogenesis that occurs with triploidy has been shown to result in increased growth rates (Table 1.2) and maintenance of meat condition due to little or no spawning activity (Shpigel et al. 1992; Jiang et al. 1993; Nell et al. 1994). This maintenance of meat condition is of benefit to differing markets. Triploid oysters have been developed in the USA, where diploids in spawning condition are less marketable and in Australia where the drop in meat condition during the cooler months traditionally created a lull in oyster sales.

Superior growth and survival of triploid over diploid oysters has also been attributed to 'hybrid vigour', particularly in meiosis I triploids which are more heterozygous than either meiosis II or diploid oysters (e.g. Beaumont and Fairbrother 1991; Hawkins et al. 1994; Hawkins et al. 2000). However, Magoulos et al.(2000) recently showed that meiosis I triploids are not always more polymorphic than meiosis II triploids. Zouros et al. (1996) suggested that recessive genes responsible for slow growth are less likely to be unmasked in polyploid shellfish, providing another possible reason for the faster growth of triploids. Guo et al. (1996, 2001) propose that polyploid gigantism, caused by an increased cell volume of triploids compared to diploids and lack of cell number compensation, is a major reason for the faster growth of triploid bivalves.

Triploid oysters (*C. gigas*) were first produced on a commercial scale in 1986 in the USA (Chew 2000). 15 years later, triploid Pacifics made up 25-50% (10-15 billion eyed larvae) of the oyster production from the larger west coast hatcheries (Chew 2000). Triploids were traditionally produced in the USA using chemical induction with cytochalasin B; however, in 1994 a new production technique was published by Guo and Allen (1994b). Several US hatcheries are now changing to this more efficient method, which uses tetraploid x diploid crosses to produce 100% triploid offspring. Globally, triploid oysters have been produced commercially in China, France, the UK and Australia with interest and research into triploids in several other countries (Chew 2000; Guo pers. comm. *in* Nell, in press). Uptake of triploids on a commercial scale in France was initially slow until 1999/2000 when tetraploid sperm was made available to hatcheries. This change in production technique is expected to increase output from 10-20% to 50% of hatchery production (Y. LeBorgne pers. comm. *in* Nell, in press). Similarly, triploids have not previously been successful in the UK but the use of tetraploid sperm is expected to stimulate further trials with triploid Pacific oysters (J. Bayes pers. comm. *in* Nell, in press).

Table 1.2

Growth advantage in terms of whole weight of meiosis II triploid bivalves

Species	Start weight (g) diploid	Start weight (g) triploid	Duration of experiment (months)	Growth advantage (whole weight)	Reference
Argopecten irradians	(shell height 26 mm)	(shell height 27 mm)	4	36%[3]	Tabarini 1984
Chlamys nobilis	-[2]	-[2]	14	18%	Komaru & Wada 1989
Crassostrea gigas	-[2]	-[2]	5	28%	Chao et al. 1999
Crassostrea gigas	-[2]	-[2]	-	46-93%	Landau & Guo 1999
Crassostrea gigas	<1	<1	27	23%	Maguire et al. 1994a
Crassostrea gigas	4	6	9	40-90%	Akashige & Fushimi 1992
Crassostrea virginica	(shell height 25 mm)	(shell height 25 mm)	6	40%	Scarpa et al. 1996
Crassostrea virginica	<1	<1	15	91%	Matthiessen & Davis 1992
Ostrea edulis[1]	-[2]	-[2]	15	47%, meiosis I triploids -12%, meiosis II triploids	Hawkins et al. 1994
Pinctada martensii	-[2]	-[2]	24	44%, meiosis I triploids 18%, meiosis II triploids	Jiang et al. 1993
Saccostrea glomerata	<1	<1	29	39%	Nell et al. 1994
Tapes philippinarum	(shell height 15-20 mm)	(shell height 15-20 mm)	48	26%	Utting et al. 1996

[1]total dry weight
[2]information not available
[3]body tissue only

One of the most efficient means of producing triploid oysters is by exposing fertilised eggs to the fungal metabolite, cytochalasin B immediately prior to the normal extrusion of the meiosis II polar body. Cytochalasin B affects the band of actin filaments that normally form the cleavage furrow between the polar body and zygote; consequently, the chromosomes of the second polar body are retained in the zygote (Longo et al 1993). Meiosis I triploids are more difficult to produce than meiosis II triploids probably due to two reasons: 1) the greater dependence on synchronisation of embryonic development and 2) the greater likelihood of producing aneuploids due to the passage of meiosis I treated embryos through meiosis II (Gerard et al. 1999).

Research in recent years has been directed at finding an alternative to the toxic chemical cytochalasin B with particular attention paid to the potential of tetraploid oysters (4n=40 chromosomes) (Fig. 1.3; Guo and Allen 1994b; Cai and Beaumont 1996). Crossing tetraploid and diploid oysters produces 100% triploid oysters (Guo et al. 1996) that may have faster growth rates than MII triploids produced using cytochalasin B (Wang et al. 1999). By producing 100% triploids, meat condition remains relatively constant within a batch of oysters. In addition, the virtual sterility of all triploid oysters opens up the possibility of translocating non-native species (Allen 2001). However, reliable artificial production of tetraploids has proven to be a difficult task with poor viability of treated embryos regardless of the method used. Several methods have been tested for production of tetraploid Sydney rock oysters (Nell et al. 1998); these included blocking both polar bodies in fertilised diploid eggs (Scarpa et al. 1993), electrofusion and blocking the first polar body in eggs from triploids fertilised by sperm from diploids (Guo and Allen 1994b). All techniques successfully produced tetraploid larvae, however, no tetraploids survived beyond metamorphosis.

The method of Guo and Allen (1994b) was refined by Eudeline et al. (2000) for Pacific oysters to produce on average 45% tetraploids at settlement with 4.4% survival to 8 days. Despite the low numbers of tetraploids produced by this method, those that do survive can be successfully crossed to produce further generations of tetraploids (X. Guo pers. com. 2000 *in* Nell, in press). Production of 100% triploid Pacific oysters is now practised in both France (J. Mazurie pers. com. 2000 *in* Nell, in press) and the USA (S. Cudd pers. com. 2000 *in* Nell, in press) by crossing tetraploid and diploid oysters. Production of tetraploid Eastern oysters using the same method has, as with Sydney rock oysters, proven a difficult task. After numerous attempts, a batch of tetraploid Eastern oysters was produced in 1999 (Supan et al. 2000).

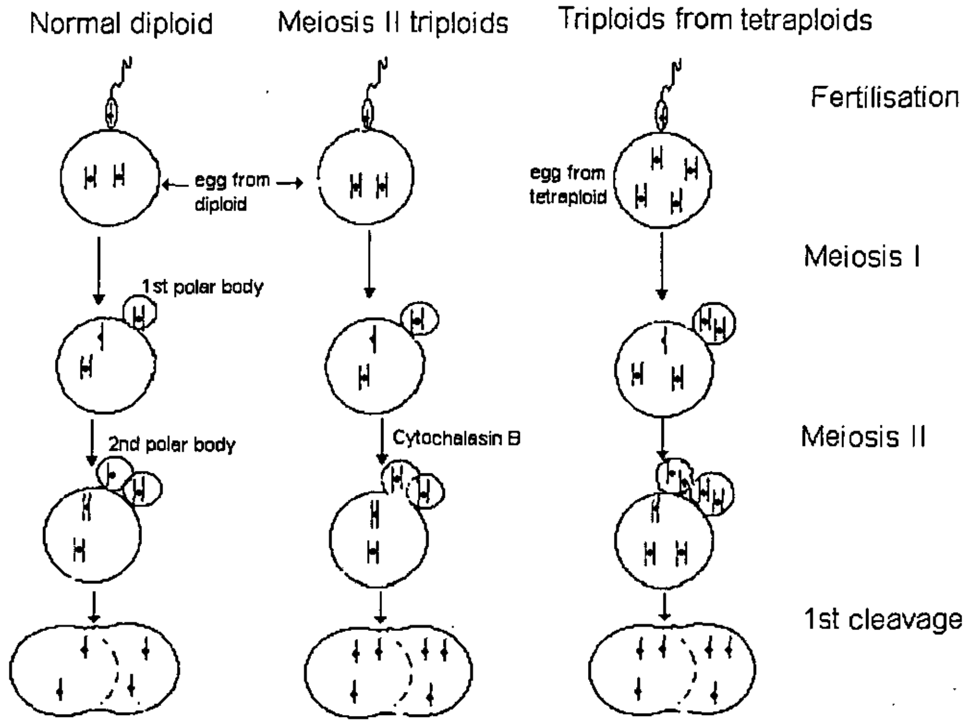

Figure 1.3 Two alternative means of producing triploid oysters - by blocking polar body II (meiosis II triploids) or from tetraploid/diploid crosses.

Normal diploid: At fertilisation the egg is at metaphase 1. Activation by the sperm results in 2 reduction divisions of the eggs' genetic material, i.e. half the chromosomes are extruded from the egg twice, in 2 polar bodies. The resulting zygote is diploid.

Meiosis II triploids: The 1st polar body removes half the chromosome number as for the diploid. Exposure of the egg to cytochalasin B following 1st polar body extrusion blocks cleavage of the 2nd polar body from the egg. The extra set of chromosomes retained by the egg results in a triploid zygote.

Triploids from tetraploids: Fertilisation by a diploid male results in similar sequence of events to diploid egg development with the exception that the tetraploid egg has double the number of chromosomes. (Adapted from Beaumont and Fairbrother 1991).

There are numerous and conflicting reports on the relative survival of diploids and triploids (Barber and Mann 1991; Meyers et al. 1991; Mathiessen and Davis 1992). Several authors have proposed that the greater heterozygosity of triploids may result in enhanced performance over diploid oysters due to 'hybrid vigour' (eg. Beaumont and Fairbrother 1991; Hawkins et al. 1994). In an earlier study on triploid Sydney rock oysters in Woolooware Bay, NSW (Nell et al. 1994), a comparison of mature diploids and triploids showed no significant difference in mortality over a winter/spring season (May-December 1992). Woolooware Bay is an area where oysters are frequently affected by the disease winter mortality and oysters in that experiment displayed signs typical of the disease.

1.5.1 Triploid oysters in Australia

In Australia, commercial scale edible oyster hatcheries are largely restricted to Tasmania and more recently South Australia (SA). Despite the large oyster industry in NSW, the low cost of naturally caught spat, unfamiliarity of many farmers with single seed technology and problems with hatchery production of Sydney rock oysters have so far prevented the successful establishment of a commercial bivalve hatchery in this state. Successful commercial scale evaluation of triploid Sydney rock oysters would hopefully encourage the establishment of a commercial hatchery in NSW. Limited quantities of triploid Pacific oysters have been produced by hatcheries in Tasmania for the Tasmanian and South Australian industries over the past 10 years. However, the Australian oyster farming industry has been slow to adopt triploid oysters. For example, less than 2% of production from the largest hatchery in Tasmania is targeted to triploid production for farmers in Tasmania and SA (R. Pugh pers. comm. *in* Nell, in press). This slow uptake is predominantly due to the limited production by hatcheries, the higher cost of triploid seed and the lack of research or information on the performance of triploid oysters across the wide range of environmental conditions experienced in Australian estuaries. Compared to triploid *C. gigas* at lower latitudes, the growth improvement in Tasmania and SA is relatively small. Meat discolouration (Maguire et al. 1994a) may also be partially responsible for limited interest in triploid *C. gigas* in Australia. The absence of a commercial hatchery in NSW has limited production in this state to small quantities for research purposes only. Nell et al. (1994) compared the performance of triploid and diploid Sydney rock oysters in a small-scale trial in a single estuary (Port Stephens) over two and a half years. Although results of the study were encouraging, large variation occurred between the four sites tested within the one estuary (range 32-48.8% difference in whole weights of diploids and triploids). Triploid Sydney rocks were on average 41% heavier, reached market size 6-18 months earlier and maintained higher condition indices than their diploid siblings (over the last 10 months of culture). The magnitude of the difference in condition index between diploids and triploids varied among leases within Port Stephens. Similarly, condition indices of diploid and triploid Pacific oysters *Crassostrea gigas* at three sites in Tasmania varied with consistently lower triploid than diploid condition indices at one site (Maguire et al. 1994b).

1.5.2 The present study: commercialisation of triploid Sydney rock oysters

Oyster farming in NSW is practised throughout the coastal zone across a range of environmental conditions. At the conclusion of the preliminary study by Nell et al. (1994) there were no commercial bivalve hatcheries operating in NSW. To commercialise production and farming of triploid Sydney rock oysters, potential hatchery operators and farmers needed to be assured that the benefits of triploidy could be extended to other oyster growing estuaries, using commercial farming methods. Triploid culture in NSW would also require adoption of small single seed culture techniques with which many farmers are unfamiliar. In this study, to evaluate the commercial potential of triploid oysters, growth and mortality (including susceptibility to winter mortality) (chapters 3 and 4) and meat condition (chapter 6) were compared between sibling diploid and triploid Sydney rock oysters grown on oyster farms throughout NSW. Culture of triploid oysters on commercial farming leases had the added benefit of providing farmers throughout the state with experience in triploid and small single seed culture as well as stimulating interest in the technology.

Production of triploids requires careful control of egg fertilisation and zygote development. This necessitates use of bivalve hatchery technology. Techniques for optimising hatchery production of triploid Sydney rock oysters were refined by Nell et al. (1996). Hatchery production of bivalve spat results in a range of size grades from each batch. Commercial hatcheries regularly grade spat and sell them at a rate according to size grade. These prices take into account the time and cost of growing a smaller grade to the same size as a larger grade as well as the concept that smaller grades may be intrinsically slower growers. The question arises as to whether a smaller grade of oysters can grow at the same rate as a larger grade of oysters. As triploids can only be produced under hatchery conditions, the question of whether different grades are of equal value in terms of growth potential is important to their commercialisation. In addition, a significant factor for commercial hatcheries to consider in triploid production is the relative growth potential of small grade triploids compared to all diploid grades. That is, it is possible that the growth advantages (due to limited gonadogenesis) of even small grade triploids may result in faster growth rates than all diploid grades. In chapter 5, growth potential of different size grades according to ploidy level (not previously examined in bivalves) is investigated for diploid and triploid Sydney rock oysters in NSW.

Selective breeding of Sydney rock oysters in Port Stephens, has produced, on average, oysters that are 18% heavier than controls. The most promising selection line "L2", was 23% heavier than controls after 18 months grow-out (Nell et al. 1999). Bayne et al. (1999) showed that L2 oysters had higher ingestion rates and lower cost of growth when compared to control oysters. As the growth advantages of L2 selected oysters and triploids are achieved through different pathways it is possible that combining the two technologies would have an additive effect on growth. In chapter 7, the performance of oysters produced using a combination of selective breeding and triploidy is evaluated.

In summary, triploid Sydney rock oysters will be evaluated according to the following criteria:

1. Growth and mortality at a range of commercial farming sites throughout

NSW (chapter 3)

2. Susceptibility to the disease winter mortality at commercial farming sites (chapter 4)

3. Growth of different size grades (chapter 5)

4. Meat condition (chapter 6).

5. Performance of selection line triploids (chapter 7).

Chapters 3 –6 are based on previously published articles and are presented in the form in which they were prepared for publication. This has resulted in some planned repetition of material in some sections. Publication details are as follows:

Chapter 3: Hand, R. E., J. A. Nell & G. B. Maguire. 1998. Studies on triploid oysters in Australia. X. Growth and mortality of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *J. Shellfish Res*. 17 (4): 1115-1127.

Chapter 4: Hand, R. E., J. A. Nell, I. R. Smith and G. B. Maguire. 1998. Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters (*Saccostrea commercialis* (Iredale and Roughley)) through outbreaks of winter mortality caused by *Mikrocytos roughleyi* infestation. *J. Shellfish Res*. 17(4): 1129-1135.

Chapter 5: Hand, R. E., J. A. Nell, D. D. Reid, I. R. Smith and G. B. Maguire. 1999. Studies on triploid oysters in Australia: effect of initial size on growth of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *Aquaculture Res*. 30: 35-42

Chapter 6: Published as: Hand, R. E. and J. A. Nell. 1999. Studies on triploid oysters in Australia. XII. Gonad discolouration and meat condition of diploid and triploid Sydney rock oysters (*Saccostrea commercialis*) in five estuaries in New South Wales, Australia. *Aquaculture* 171: 181-194.

2 METHODS FOR PRODUCTION OF TRIPLOID SPAT AND DETERMINATION OF PLOIDY IN SYDNEY ROCK OYSTERS

2.1 INTRODUCTION

Of the numerous methods (excluding tetraploids) available for induction of triploidy in oysters (discussed in the review of Beaumont and Fairbrother (1991)) the greatest yield of triploids is generally achieved by use of the fungal metabolite cytochalasin B (CB) to block extrusion of the second polar body at meiosis II. Triploids can be produced by blocking extrusion of either the first (meiosis I triploids) or second (meiosis II triploids) polar body using either physical or chemical stress. Physical stress, using either temperature (Toro et al. 1995; Toro & Sastre 1995) or pressure (Komaru & Wada 1989), yields low and unreliable numbers of triploids. Scarpa et al. (1994) compared six methods (CB, heat, calcium, caffeine, calcium combined with heat and caffeine combined with heat) for producing triploid mussels, *Mytilus galloprovincialis.* Yield of triploid larvae after 96 hours was highest in the CB group, followed by the heat shock group. Survival (<50%) was poor in the remaining treatments reducing the overall yield of triploids.

Nell et al. (1996) investigated two of the more promising chemicals for induction of meiosis II triploidy in Sydney rock oysters, CB and 6-dimethylaminopurine (6-DMAP). Although triploid Sydney rock oyster larvae were obtained with 6-DMAP, the highest day 5 yield of triploids was achieved by exposure of eggs to either 1 or 1.25 mg/l CB at 50% first polar body extrusion. For all Sydney rock oyster commercialisation experiments, other than the one described in chapter 7, oysters